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**The combined effect of ALK inhibitors and radiotherapy in non-small cell lung cancer**

Promotionsfach: Radiologie
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ALK activation via EML4-ALK-fusion is found in approximately 2-7% of NSCLCs patients. Patients with EML4-ALK positive lung cancer are sensitive to ALK-kinase inhibitors. Crizotinib and TAE684 are two potent ALK tyrosine kinase inhibitors. Crizotinib has been recently approved and replaced chemotherapy in second line treatment of advanced EML4-ALK positive NSCLC. TAE684 is a second-generation ALK inhibitor overcoming Crizotinib resistance. Radiotherapy is an integral component of treatment for locally advanced lung cancer. Here we sought to investigate the effects of combined radiotherapy and ALK inhibition via TAE684 and Crizotinib. The radiosensitizing potential of Crizotinib and TAE684 were investigated *in vivo* and *in vitro* in NSCLC cell lines with EML4-ALK translocation H3122, and wild type A549 and LLC cells. Cells were irradiated with 1-4 Gy X-Rays and carbon ions at Heidelberg Ion Therapy center. TAE684 was administrated at the dose range 0-100nM and Crizotinib was administrated at the dose range 0-200nM. The clonogenic survival of cells exposed to a dose series of radiotherapy and ALK inhibitors was evaluated in all three cell lines. Fluorimetric measurements and time-lapse life microscopy were performed to detect cell proliferation as well as apoptosis (caspase 3/7 activation). The syngeneic mouse (LLC) and human (H3122) xenograft tumor models were further studied *in-vivo* with the following assessment of tumor growth kinetics, microvascular density (MVD), perfusion and proliferation. ALK inhibitors inhibited the proliferation of H3122 cells in a dose-dependent manner ($IC_{50}$: TAE684: 8.2nM; Crizotinib: 84nM). However, A549 and LLC cells were relatively resistant to ALK inhibitors and the $IC_{50}$ was not reached at concentrations tested (~100nM for TAE684 and ~200nM for Crizotinib) in proliferation assay. The antiproliferative effect of TAE684 and Crizotinib was augmented by radiotherapy in H3122 cells. A synergistic effect of combined ALK inhibitors and radiotherapy in ALK-positive NSCLC was indicated by isobologram analysis based on clonogenic survival data. Moreover, ALK inhibitors significantly sensitized H3122 cells to particle therapy with carbon

ions (SER: TAE684: 1.92; Crizotinib: 1.49 $p<0.05$). Caspase 3/7 activity was enhanced by combination therapy in H3122 cells up to 3-folds compared with monotherapy with ALK inhibitors. In H3122 xenografts, dual combination was more effective in reducing tumor proliferation, MVD and perfusion. In contrast, in the LLC model, Crizotinib led only to a transient tumor growth inhibition and combined treatment was inferior to radiotherapy alone. In this project, we demonstrated the synergistic effects of combined TAE684 or Crizotinib and radiotherapy in EML4-ALK positive lung cancer cells. In addition to conventional photon radiotherapy, ALK-inhibition also enhanced the effects of particle irradiation using carbon ions. In parallel, the *in-vivo* data supports the beneficial effects of combination therapy only in ALK positive NSCLC tumors. Our data equivocally indicate beneficial effects of combined ALK-inhibition and radiotherapy in ALK-fusion harboring NSCLC. Therefore, this study constitutes a critical step towards clinical translation of this favorable combination in NSCLC.